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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 4534-4538

www.thepharmajournal.com
Received: 13-04-2022
Accepted: 16-05-2022

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Correlation of some blood metabolites with THI in lactating Murrah buffaloes during one lactation

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Abstract
The study aimed to evaluate and correlate metabolites and certain enzymes with Temperature Humidity Index (THI) during one complete lactation and in Murrah buffaloes. Blood sampling was done on 7th and 15th day of parturition and thereafter at fortnightly intervals till 210 days of lactation. The animals were neither artificially inseminated nor allowed to mate throughout the study. All the samples were procured as per the standard procedure. The result indicated that the concentrations of serum urea, creatinine, serum glutamate – pyruvate transaminase, serum glutamic – oxaloacetic transaminase, glucose, differed significantly ($P<0.01$). Of the four studied serum electrolytes phosphorus and chloride were found non-significant. The correlation studies with THI were not significant for the serum biochemical parameters.

Keywords: Buffaloes, biochemical, enzymes, lactation, temperature humidity index

1. Introduction

Buffalo is predominantly dairy animals distributed in different regions of the country and is well adapted to the local agro-climatic conditions (Garkal *et al.* 2016) [8]. In tropical and subtropical regions, the summer is prolonged, characterized by high environmental temperatures and humidity, producing high THI and heat stress on animals, altering physiological, metabolism, production, reproduction, and energy balance (Wankar *et al.* 2020) [31]. The quantification of blood metabolites is a handy guide for the animals' nutritional status and potential performance (Garcia *et al.* 2017) [7]. The blood urea nitrogen (BUN), a strategic marker of the animal's energy intake, elicits the synchronization between fermentable carbohydrates and rumen degradable protein (Van Saun 2010) [30]. The renal functions, principally embodied by urea and creatinine concentrations, are significantly affected during the different physiological phases. An imperative yardstick of a ruminant's energy status is its glucose concentration which is expended by the mammary gland and Ingvartsen *et al.* (2003), Ingvartsen (2006) and LeBlanc (2010) [14, 15, 20] have identified plasma glucose as the foremost metabolite that correlates to the intensity of physiological imbalance.

The enzymes SGPT / ALT (serum glutamate-pyruvate transaminase / alanine aminotransferase) and SGOT / AST (serum glutamic-oxaloacetic transaminase / aspartate aminotransferase) are vital catabolic enzymes that play a critical role in the liver function of animals (Bjerre-Harpoth *et al.* 2012) [4]. Electrolytes have multifaceted functions in an animal's body like incorporating plasma osmolarity conservation, acid-base equilibrium, nerve impulse propagation. They are co-factors for various enzymes, thus playing a decisive role in sustaining metabolic homeostasis (Jacob *et al.* 2011) [16]. The status of macro minerals like calcium (Ca), phosphorus (P), and magnesium (Mg), due to their significance in the metabolic reactions expeditiousness and their role in the transmembrane transport systems (Houillier 2014) [12] along with chloride (Cl) the most abundant anion in extracellular fluid are of critical relevance because of their role in metabolic challenges like ketosis and milk fever wherein they play a crucial role and alterations commonly occur in the body around parturition and during peak lactation.

Livestock performance is influenced by different elements due to complex interactions between the individual animal and the different environmental factors (Lambertz *et al.* 2013) [19]. One of the greatest challenges confronted by domestic animals around the world is thermal stress, which, in the tropics, is the chief limiting factor in livestock production. It has a decidedly unfavorable consequence on the animal bioenergetics, performance and health. The most crucial influencers of heat stress are temperature and humidity (Bohmanova *et al.* 2005) [5]. There are many techniques for assessing the thermal load, and one of the most efficacious is gauging the temperature humidity index (THI) that combines dry bulb and wet bulb temperatures along with relative humidity to quantify heat stress (Thom, 1959) [29]. Behera *et al.* (2018) [3] identified the THI model developed by NRC (1971) as the most suitable temperature humidity index (THI model) to study the impact of thermal stress. In terms of THI, the values of THI >72 is considered as stressful and THI >78 is considered traumatic for dairy cows and buffaloes (Ganaie *et al*., 2013).

2. Materials and Method

2.1 Animal selection and experimental design

Fifteen apparently healthy and about to parturate Murrah buffaloes who were in their 2nd – 4th lactation were included in the study. The buffaloes were between 5 – 7 years of age and their average milk yield was 8 – 16 liters per day. The animals were neither artificially inseminated nor allowed to mate throughout the study.

2.2 Blood sampling

Blood samples were collected aseptically, just after milking, on 7th and 15th day of parturition and after that at fortnightly intervals (days 30th, 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th, 180th, 195th and 210th of lactation) till 210 days of lactation (drying off) from the same buffaloes throughout their lactation by jugular vein puncture into serum clot activator tubes to separate the serum.

2.3 Sample analysis

The serum urea, creatinine, glucose, glutamate pyruvate transaminase, glutamic oxaloacetic transaminase, calcium, phosphorous, chloride and magnesium were analysed using standard kits on the autoanalyser.

2.4 Calculations of temperature humidity index

The Temperature Humidity Index (THI) was calculated by using the National Research Council formula THI = $(T_{db} + T_{wb}) \times 0.72 + 40.6$

2.5 Statistical analysis

The data was analyzed by complete randomized design using WASP – 2 (Web Agri Stat Package), ICAR, differences in means were tested using the critical difference (CD) test.

3. Results and Discussion

3.1 Serum urea

The mean serum urea concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 01. It was perceived that urea concentration varied significantly (P<0.01) throughout the lactation. The mean values gradually augmented till day 90 and thereafter declined till the end of the lactation (day 210), which is in accordance with the findings reported by Naser *et al.* (2014) [22] in the dairy cow. The source of blood urea is either excess protein catabolism or deamination of amino acid during lactational stress and due to pathways of milk biosynthesis. An increase may indicate extra protein breakdown or mobilization for milk constituent synthesis. The stress of parturition and early lactation may increase cortisol secretion, causing protein breakdown and increased hepatic deamination (McDonald 1980). The blood urea levels were higher when the THI was elevated, which supports the findings of Shwartz *et al.* (2009) [27] in heat-stressed cows during mid-lactation.

3.2 Serum creatinine

The mean serum creatinine concentrations analyzed during the study are reported in Table 01. The data analysis revealed that the creatinine concentration was higher during early lactation and remained steady till mid-lactation (day 90) with little fluctuation. The concentration decreased significantly (P<0.01) until the end of the study. Similar findings were also reported by Yadav *et al.* (2016) [32] during heat stress in Murrah buffaloes, which could be due to increasing THI that induced the heat stress. During heat stress, protein breakdown may occur for gluconeogenesis to suffice energy demands for thermoregulation. In the present research, the elevated early lactation serum creatinine levels could be associated with the physiological status of increasing milk yield and surrounding climatic factors triggering a surge in the THI.

3.3 Serum glucose

The mean serum glucose concentrations throughout lactation are represented in Table 01. The analysis indicated a significant (P<0.01) but moderate elevation during early and mid-lactation. Serum glucose concentrations were troughed on days 30, 45, 90 and 105 and crested on 150, 165 and 180 days of lactation. Overall, the levels were marginal immediately after parturition at the beginning of lactation and augmented thereafter. Early lactation was marked with subsided and mid lactation by surging serum glucose concentrations in the present study which is in accordance with Naser *et al.* (2014) [22]. The stress of parturition and utilization of serum glucose for milk biosynthesis can be attributed to low serum glucose on day 07 postpartum. This is also significant as glucose delivery and uptake by mammary gland is the rate-limiting step of milk synthesis. In our study, the serum glucose values maintained an inverse relationship with THI wherein it was perceived that whenever the THI was high, serum glucose was low. Studies done by Guo *et al.* (2018) [10] are in concurrence with our finding of heat stress lowering blood glucose.

3.4 Serum glutamate – pyruvate transaminase

The mean serum glutamate – pyruvate transaminase / alanine aminotransferase (SGPT / ALT) concentrations in the investigated lactating Murrah buffaloes are posted in Table 01. In the present exploration, the SGPT values were found to be significantly (P<0.01) high during early and late lactation but low during mid-lactation. Analogous to ours are the findings reported by Ashmawy *et al.* (2015) [1] which revealed low levels of SGPT occurring on day 60, but the lowest was on day 210 and they reasoned that the SGPT activity surge in buffalo blood during lactation signified an intensification of hepatic metabolism and the variations in its enzymatic activities may be due to decreased dry matter intake that subsequently impacts hepatic lipidosis to generate altered liver function. The effect of THI on SGPT levels appear to be better associated during early lactation as high milk yield is likely to be affected more as compared to low milk production. Early lactation is characterized by increasing milk yield up to peak lactation. Higher SGPT values due to summer or heat stress has also been reported by Kalamath (2015) [17].

3.5 Serum glutamic oxaloacetic transaminase

The mean serum glutamic – oxaloacetic transaminase / aspartate aminotransferase (SGOT / AST) concentrations from day 7 to day 210 of lactation as represented in Table 01 reveal that the mean values of AST were significantly (*P*<0.01) higher in early and mid-lactation with minor fluctuation as compared to late lactation, which supports the findings of Ghanem *et al.* (2012). High SGOT concentrations during early lactation immediately after parturition are also exemplified by colostrum synthesis that necessitates improved metabolic rate and induces enhanced catabolism for maximum production. During the mid-lactation the declining levels of SGOT could be elucidated by the optimum care and nutrition given to the lactating dam. Moreover, around mid-lactation, the stress of augmenting milk yield also tempered it down. In the present research, THI increase was associated with increased AST levels even though it had an additive effect and early lactation stress. A significant increase in AST levels due to increase in THI during heat stress has been observed by Kalamath (2015) [17] in his study.

Table 1: Serum urea, creatinine, ALT, AST, glucose from day 7 to day 210 of lactation in lactating Murrah buffaloes

Days of lactation	Urea (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	SGPT (IU/L)	SGOT (IU/L)
07	30.85^{cd} ± 3.30	1.40^{a} ± 0.06	40.15^{h} ± 2.12	37.03^{def} ± 1.53	165.80^{a} ± 10.53
15	36.61^{bc} ± 2.88	1.33^{a} ± 0.07	62.81^{def} ± 4.58	42.69^{cde} ± 1.87	151.8 ^{abc} ± 4.04
30	30.30^{cd} ± 1.51	1.23^{ab} ± 0.06	51.36^{fgh} ± 5.35	38.03^{def} ± 1.61	157.43^{ab} ± 6.39
45	32.66^{cd} ± 2.38	1.40^{a} ± 0.09	50.62^{gh} ± 5.41	40.30^{de} ± 1.76	159.57^{ab} ± 5.75
60	32.36^{cd} ± 1.36	1.26^{ab} ± 0.06	71.03^{bcd} ± 5.92	49.90^{abc} ± 3.04	140.50^{bcd} ± 4.61
75	34.32^{bc} ± 1.63	1.34^{a} ± 0.08	56.05^{efg} ± 4.63	50.84^{ab} ± 1.39	137.80^{cde} ± 5.76
90	45.70^{a} ± 3.71	1.05^{bc} ± 0.06	48.53^{gh} ± 3.72	54.59^{a} ± 3.42	121.23^{de} ± 7.77
105	40.98^{ab} ± 4.15	0.64^{fg} ± 0.12	50.10^{gh} ± 3.48	43.51^{bcde} ± 1.26	129.21^{de} ± 4.23
120	46.71^{a} ± 4.77	0.93^{cde} ± 0.07	66.41^{de} ± 3.47	44.18^{bcd} ± 1.78	154.55^{abc} ± 7.33
135	41.03^{ab} ± 4.36	0.44^{g} ± 0.10	67.17^{cde} ± 5.17	24.50^{h} ± 2.59	121.51^{de} ± 13.97
150	31.99^{cd} ± 3.27	1.00^{cd} ± 0.06	81.70^{ab} ± 5.75	26.52^{h} ± 4.51	36.18^{h} ± 3.76
165	34.52^{bc} ± 2.04	0.78^{ef} ± 0.07	90.60^{a} ± 3.17	32.22^{fgh} ± 2.54	131.57^{de} ± 4.04
180	25.31^{de} ± 1.13	0.56^{g} ± 0.04	86.89^{a} ± 3.18	28.98^{gh} ± 2.94	119.65^{e} ± 6.87
195	19.47^{ef} ± 0.71	0.19^{h} ± 0.01	45.13^{gh} ± 3.26	36.18^{efg} ± 4.86	82.20^{f} ± 6.63
210	16.89^{f} ± 1.21	0.83^{def} ± 0.12	78.87^{abc} ± 2.80	28.43^{gh} ± 3.78	61.39^{g} ± 5.28

Mean values within a column with no common superscript differed significantly (*P*<0.01)

3.6 Serum calcium

The mean serum calcium (Ca) concentrations in lactating Murrah buffaloes are presented in Table 02. They showed a significant (*P*<0.01) high on day 7 of lactation, which was followed by a decrease in the serum calcium levels. From day 60 to day 105 of lactation the serum calcium concentration surged non-significantly. Subsequently, there was a small dip and followed by a rise upto day 150 of lactation. Thereafter, the concentration waned till a sudden rise on day 210 of lactation. During our investigation, there was a drop in calcium level during the early stage of lactation, which corroborates with the findings of Kashwa (2016) [18] in lactating buffaloes. Maternal mineral and skeletal homeostasis during lactation is governed by the need of calcium for milk production. During early lactation, the lower calcium levels may be because of voluminous calcium demand and inadequate parathormone secretion (Paul *et al.* 2011) [25]. The metabolic and physiological status of the animal governs its ability to uptake and use the available calcium.

Table 2: Serum Ca, P, Cl and Mg and THI from day 7 to day 210 of lactation in lactating Murrah buffaloes

Days of lactation	Ca (mg/dl)	P (mg/dl)	Cl (mEq/L)	Mg (mg/dl)	THI
07	10.65^{abc} ± 0.43	6.22^{NS} ± 0.40	101.03^{NS} ± 2.13	2.44^{bc} ± 0.08	74.94
15	09.19^{efg} ± 0.36	5.61^{NS} ± 0.37	99.93^{NS} ± 1.38	2.30^{cd} ± 0.13	75.52
30	08.30^{g} ± 0.44	5.57^{NS} ± 0.41	100.13^{NS} ± 4.98	1.88^{fg} ± 0.11	78.33
45	10.15^{bcde} ± 0.43	6.09^{NS} ± 0.35	100.61^{NS} ± 3.47	2.32^{cd} ± 0.12	83.08
60	11.55^{a} ± 0.48	6.28^{NS} ± 0.24	98.08 ^{NS} ± 5.21	2.09^{def} ± 0.14	80.63
75	10.49^{abc} ± 0.31	5.39^{NS} ± 0.34	96.52^{NS} ± 1.29	2.4^{c} ± 0.08	81.57
90	11.18^{ab} ± 0.46	5.45^{NS} ± 0.23	87.28^{NS} ± 2.14	2.79^{a} ± 0.09	83.08
105	10.71^{abc} ± 0.45	5.39^{NS} ± 0.30	97.63^{NS} ± 1.81	2.01^{efg} ± 0.09	85.96
120	09.74^{cdef} ± 0.38	5.67^{NS} ± 0.29	98.80^{NS} ± 0.59	2.08 ^{def} ± 0.08	80.20
135	11.08^{ab} ± 0.48	6.13^{NS} ± 0.29	99.26^{NS} ± 2.65	1.94^{efg} ± 0.06	82.36
150	11.52^{a} ± 0.34	5.94^{NS} ± 0.47	101.76^{NS} ± 2.12	2.33^{cd} ± 0.10	81.64
165	09.74^{cdef} ± 0.29	6.04^{NS} ± 0.29	97.57^{NS} ± 1.71	2.18^{cde} ± 0.08	77.32
180	09.25^{defg} ± 0.45	5.42^{NS} ± 0.33	97.04^{NS} ± 1.03	1.76^{g} ± 0.06	78.76
195	08.79^{fg} ± 0.39	5.38^{NS} ± 0.25	94.80^{NS} ± 1.18	2.09^{def} ± 0.09	80.20
210	10.34^{bcd} ± 0.40	5.47^{NS} ± 0.30	99.30^{NS} ± 3.01	2.70^{ab} ± 0.11	81.86

Mean values within a column with no common superscript differed significantly (*P*<0.01)
NS Non significant

3.7 Serum phosphorus

The mean serum phosphorus (P) concentrations in our research are depicted in Table 02. Non-significant differences were recorded in the serum phosphorus concentrations, although the mean values fluctuated up and down throughout the study as per the calcium concentration, which is in accordance with Hagawane *et al.* (2009) [11] in lactating buffaloes. A moderate reduction in phosphorus levels might be due to its necessity for colostrum synthesis (Serdaru *et al.* 2011) [26]. The ostensibly depressed phosphorus levels in early and mid-stage of lactation was likely in part due to its removal with milk (Valk *et al.*, 2002).

3.8 Serum chloride
The mean serum chloride (Cl) concentrations in lactating Murrah buffaloes are elucidated in Table 02. The Cl concentration differed non-significantly during whole lactation period in the present study. The values obtained in the present study is lower than the values reported by Dukes *et al.* (2015) [6]. The Cl concentrations are associated with sodium concentration; fluctuations in sodium concentration may modify the chloride concentration. According to Hu and Murphy (2004) [13], chloride concentration is especially reliant on the nutrient intake. An incorrect feeding regime could be concomitant of low levels of chloride. They may also be supplementary to intermittent offering of salt licks in conjunction with the intermittent offering of salt licks in conjunction the likelihood of chloride deficient feed. No specific trend of increase and decrease of mean values was observed when it was compared to THI.

3.9 Serum magnesium
The mean serum magnesium (Mg) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are communicated in Table 02. Our research showed more or less significant (P<0.01) variations in the Mg concentrations all through the 210 days study period. Similar trends have been reported by Nehra (2016) in dairy cows. Magnesium has a crucial role in carbohydrate, lipid, nucleic acid and protein metabolism and Mg is required for normal skeletal development and one of the most common enzyme activators (Bamerny 2013) [2]. Its apparently low level in early and mid-stage of lactation could likely be in part, due to its inclusion in the milk (Valk *et al.*, 2002). An added rationale for lower magnesium concentrations in lactating buffaloes could be attributed to its need for colostrum synthesis and superior carbohydrate metabolism (Serdaru *et al.*, 2011) [26].

3.10 Correlation Analysis with THI
The correlation analysis of effect of Temperature Humidity Index (THI) on the estimated parameters from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 03. The correlation of all the parameters with THI in the present study did not differ significantly. However, urea, Ca, Mg and SGPT were positively correlated to THI and creatinine, SGOT, P, Cl and glucose were negatively correlated with THI but the difference is non-significant.

Table 3: Pearson Correlation Coefficient (R) between the average parameters from day 7 to day 210 of lactation in lactating Murrah buffaloes and THI during that period

Parameter	(R)	Parameter	(R)
Serum urea	0.215	Serum creatinine	– 0.274
Serum SGPT	0.129	Serum SGOT	– 0.366
Serum calcium	0.484	Serum Glucose	– 0.079
Serum magnesium	0.101	Serum phosphorus	– 0.228
		Serum chloride	– 0.277

The THI during the study ranged from 74.94 at the commencement of the study to 81.86 at its termination. The maximum THI of 85.96 was noted on day 105 which fell in October. This period also coincided with that stage of lactation when the peak production had plateaued during the persistency period. Since during this stage there is already a moderating effect of the milk production and the buffaloes were therefore not in a huge energy deficit, it is likely that the actual effect of THI was not reflected.

4. Conclusion
Thus, it is concluded that, all the metabolites and enzymes altered significantly except phosphorus and chloride, whereas, its correlation with THI was not significant. The obtained results affirmed that the lactation period is accompanied by marked changes in most of the studied biochemical variables and hormones in the buffaloes. The main advantage of this study was twofold; all the animals selected belonged on the same farm and were housed in the same shed, thus reducing the variables and they were all kept open till drying. So the results reflected only the lactational and environmental developments negating variables like pregnancy metamorphoses and other sundry influences. These results can be used as a reference yardstick for non-pregnant lactating buffaloes during one complete lactation.

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